OLIG gene targeting in human pluripotent stem cells for motor neuron and oligodendrocyte differentiation.

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Public Summary:
Pluripotent stem cells can be genetically labeled to facilitate differentiation studies. In this paper, we describe a gene-targeting protocol to knock in a GFP cassette into key gene loci in human pluripotent stem cells (hPSCs), and then use the genetically tagged hPSCs to guide in vitro differentiation, immunocytochemical and electrophysiological profiling and in vivo characterization after cell transplantation. The Olig transcription factors have key roles in the transcription regulatory pathways for the genesis of motor neurons (MNs) and oligodendrocytes (OLs). We have generated OLIG2-GFP hPSC reporter lines that reliably mark MNs and OLs for monitoring their sequential differentiation from hPSCs. The expression of the GFP reporter recapitulates the endogenous expression of OLIG genes. The in vitro characterization of fluorescence-activated cell sorting-purified cells is consistent with cells of the MN or OL lineages, depending on the stages at which they are collected. This protocol is efficient and reliable and usually takes 5-7 months to complete. The genetic tagging-differentiation methodology used herein provides a general framework for similar work for differentiation of hPSCs into other lineages.

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